OPEN ACCESS J. Arab. Crops Market. 01 (02) 2019. 45-52 DOI: 10.33687/jacm.001.02.3454

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Available Online at EScience Press

Journal of Arable Crops and Marketing

ISSN: 2709-8109 (Online), 2709-8095 (Print)

https://esciencepress.net/journals/JACM

Influence of foliage applied moringa leaf extract on growth and yield of sunflower (*Helianthus annuus* L.) underwater deficit conditions

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ARTICLE INFO

Article History
Received: June 12, 2019
Revised: August 17, 2019
Accepted: December 23, 2019

Keywords
Moringa leaf extract
MLE
Protein content
Oil content
Achene yield

ABSTRACT

With increasing population, the demand for food is also increasing. So, with the aim to increase the productivity of crop a field experiment was conducted to determine the effectiveness of the foliar application of moringa leaf extract (MLE) at growth and yield of sunflower under water deficit conditions at Agronomy research area, Bahauddin Zakariya University, Multan. The treatments comprise of Factor A: Irrigation,(I_0 = Normal irrigation, I_1 = Skipped at knee height stage, I_2 = Skipped at knee height + heading stage, I_3 = Skipped at knee height + heading + seed development stage and Factor B: Foliar application of MLE (Subplot), T_0 = No spray, T_1 = Spray at knee height stage, T_2 = Spray at knee height + heading stage, T_3 = Spray at knee height + heading + seed development stage with randomized complete block design (RCBD) split-plot design in three replications. The interactive effect between moringa leaf extract (MLE) and irrigation was significant. Maximum head diameter (22.17cm), number of achenes per head (1030.30), 1000-achenes weight (49.73g), achene yield (3474 kg/ha), and biological yield (10729.83 kg/ha), oil content (41.734%), protein content (20.8%) was observed in F3 (K+H+S) and I_0 (Normal) irrigation. While minimum head diameter (15.57cm), number of achenes per head (681.67), 1000-achenes weight (42.001g), achene yield (2536.33 kg/ha), and biological yield (8209.33 kg/ha), oil content (36.3%), protein content (16.27%) was observed in F_0 (no spray) and I_3 (K+H+S) irrigation. Results showed that foliar application of moringa leaf extract under normal conditions boost the crop yield and drought stress at (K+H+S) is detrimental for growth and development of sunflower.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is ranked 4th important oilseed crop in the world after soybean, rapeseed and mustard . Pakistan is producing only 7 percent edible oil of its total consumption. In Pakistan, the sunflower is also important oilseed crop because its seed contains 40-45 % oil contents and cultivated in spring and autumn season which is helpful to overcome the annual import of edible oil . World population is increasing with the passage of time so food production must be increased to meet the need of growing population .

Crops face different environmental stress during the growing season which affects its growth and development processes . Sunflower is highly sensitive to water at early flowering and achenes filling stage . reported that drought stress reduces the achenes, oil quality and oil yield greatly. Underwater deficit condition sunflower growth is affected due to poor photosynthetic rate which is resulted by stomata closure . Low moisture in sunflower resulted declined in stomatal conductance and CO_2 assimilation . Water shortage also caused overproduction of ROS species which damage cellular components .

To overcome this water shortage in field crop production different strategies like cultural practices and breeding strategies can be adopted. Exogenous applications of natural growth regulators like auxins and osmoprotectants can also make the crop resistant to drought stress. Different synthetic and natural growth regulators are being used but natural growth regulators is gaining popularity as they are environmentally safe and cheap.

Among them, Moringa leaf extracts (MLE) has a notable position. Moringa is considered a miracle tree because it is a source of protein, vitamin C, easily digestible iron (Fe) and Ca . Moringa leaves extract produced effective growth hormones . He also resulted that it increases crop yield up to 30%. reported that moringa leaf extract enhances the crop growth, improve the resistance against pests and diseases, accelerate the size and number of fruits, number of roots and whole plant defence system.

MATERIALS AND METHODS

Preparation of Moringa leaf extract

For moringa leaf extract (MLE), young moringa (*Moringa oleifera* L.) leaves were harvested from research area of Department of Forestry, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. In extraction machine young laves of moringa were ground with water at the rate of 1L per 10kg fresh material as described by . After grinding, the material was sieved via muslin cloth and was centrifuged for 15 mints at 8000×g. The dilution of (1:30) MLE-30 (1ml MLE diluted with 30ml distilled water) of the extract was prepared as described by and this solution was used in the experiment as a foliar spray.

Experimental treatments, design and crop husbandry

Experimental treatments

This field experiment was conducted at the Research area of Department of Agronomy, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. This site lies at 30.258˚ N and 71.517˚ E. The experimental treatments were comprised of two factors, Factor-A: Irrigation (Main plot), I_0 = Normal irrigation, I_1 = Skipped at knee height stage, I_2 = Skipped at knee height + heading stage, I_3 = Skipped at knee height + heading + seed development stage, Factor B: Foliar application of MLE (Subplot), T_0 = No spray, T_1 = Spray at knee height stage, T_2 = Spray at knee height + heading stage, T_3 = Spray at knee height + heading + seed development stage.

Experimental design

The experiment was laid out in Randomized Complete Block Design (RCBD) with the split-plot arrangement was laid out in three replicates. The net plot size was $15m^2$.

Crop husbandry

For fine field bed preparation, the designed field was ploughed 2 times and tractor drew blade was used for eradication of weeds and other roots. For sowing purpose, fine structures of ridges were made at the distance of (R × R) 5 feet. A hybrid seed of (Hysun-33) was collected from the local market (Seraj Agro Services) of Multan. The crop was sown during the spring season on January 15, 2018. To attain the desired population, the optimum distance from the plant to plant was kept (P×P= 0.75 feet). A recommended dose of NPK was applied @ of 150, 100 and 60 kg/ha. The sources of artificial fertilizer include the DAP, Urea and SOP. The total amount of Nitrogen was applied into three splits, the 1/3 amount of nitrogen was applied at the time of sowing; the leftover amount of nitrogen was applied at 2^{nd} and 4^{th} irrigation. To attain the desired population, thinning was done at 3^{rd} leaf stage. Due to sensitive to hormones, the use of herbicides or weedicides was avoided. To control weeds competition, manual weeding and hoeing was done. In irrigation scheduling, first irrigation was applied after 15 days of sowing, second irrigation was applied at knee height, 3^{rd} irrigation was applied at 50 days after sowing (DAS), 4^{th} irrigation was applied at 70 days after sowing (DAS), and last irrigation was applied at the seed development stage. In drought stress treatments, as per treatments, the irrigation was skipped at knee height stage, heading stage and seed developmental stage. Foliar spray of moringa leaf extract (MLE) was sprayed at knee height, heading stage and seed development as per treatments. Crop protection measurements were also done at every stage. The sprays of Carbofuran @ 8kg/acre and Lufenuran @ 250ml/acre were sprayed at leaf stage to protect from stem borer and attack of armyworm respectively. During experiment different morphological, physiological and yield-related attributes were observed.

Table 1. Pre experiment physicochemical analysis of soil.

Determination	Value	Unit
pH	8.6	
EC	2.5	dsm^{-1}
Organic matter (O.M)	0.67	%
N	0.04	%
P	5.51	Ppm
K	192	Ppm
Sand	27	%
Silt	52	%
Clay	21	%
Soil texture	Silty clay loam	

Procedures

Leaf area index

Leaf area index was calculated by Watson (1947) formula. To calculate leaf area index, total plant in the area of $1m^2$ were harvested and their leaves were weighed immediately.

$$LAI = \frac{\text{Leaf area (cm)}}{\text{Ground area (cm)}}$$

To calculate leaf area, leaf length was multiplied by means of leaf width.

Crop growth rate (g m^{-2} day^{-1})

To determine crop growth rate, five plants from each plot were chopped after harvesting with an interval of thirty days. Chopped samples were oven dried at 70^0C and constant their dry weight to determining crop growth rate as by Hunt (1978).

$$CGR = w_2\text{-}w_1 / t_2\text{-}t_1$$

Where w_1 and w_2 are final dry matter at t_1 and t_2 respectively.

Net assimilation rate (gm^2day^{-1})

Hunt (1978) formula was used to calculate net assimilation rate.

NAR= TDM/LAD (Where TDM is total dry matter)

Achenes oil contents (%)

It was measured by Soxhlet Fat Extraction method (A.O.A.C., 1990) with the following equation.

Oil percentage= wt. of flask + oil - the weight of flask / wt. of flask + achenes-weight of flask × 100

Achenes protein contents (%)

Kjeldahl method (Bremner, 1964) was used to calculate Achene protein contents (%) by given formula.

Protein (%) = $(V_1 - V_2)$ N / 100 W × 6.25 × 14 × 100

Where, V_1 = titration sample (ml), V_2 = Blank titration (ml), N = Sulphuric acid Normality

W = Weight of sample

Weather data

Weather data was obtained from the observatory (agro-metrological department) of Central Cotton Research Institute (CCRI), Multan.

Statistical analysis

The data was analyzed to Fischer's analysis of the variance by using the Statistix 8.1. By using the LSD test with P ≤ 5% multiple the comparisons to separate means of the treatments were performed.

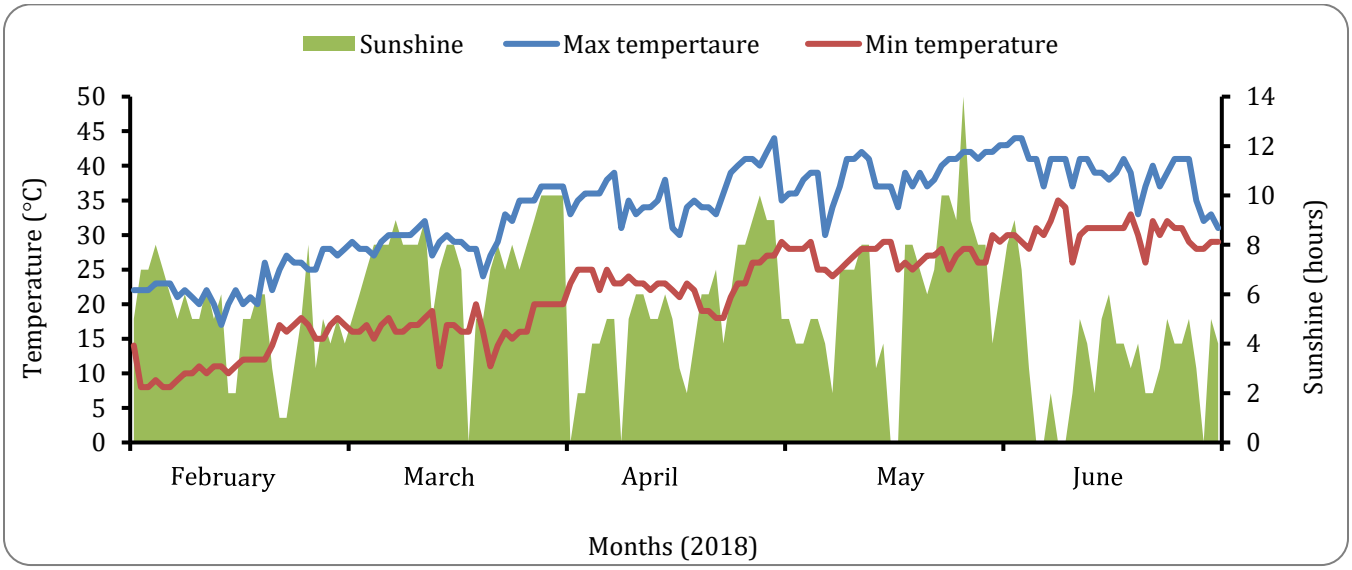

Figure 1. Meteorological data of air temperature sunshine and air temperature.

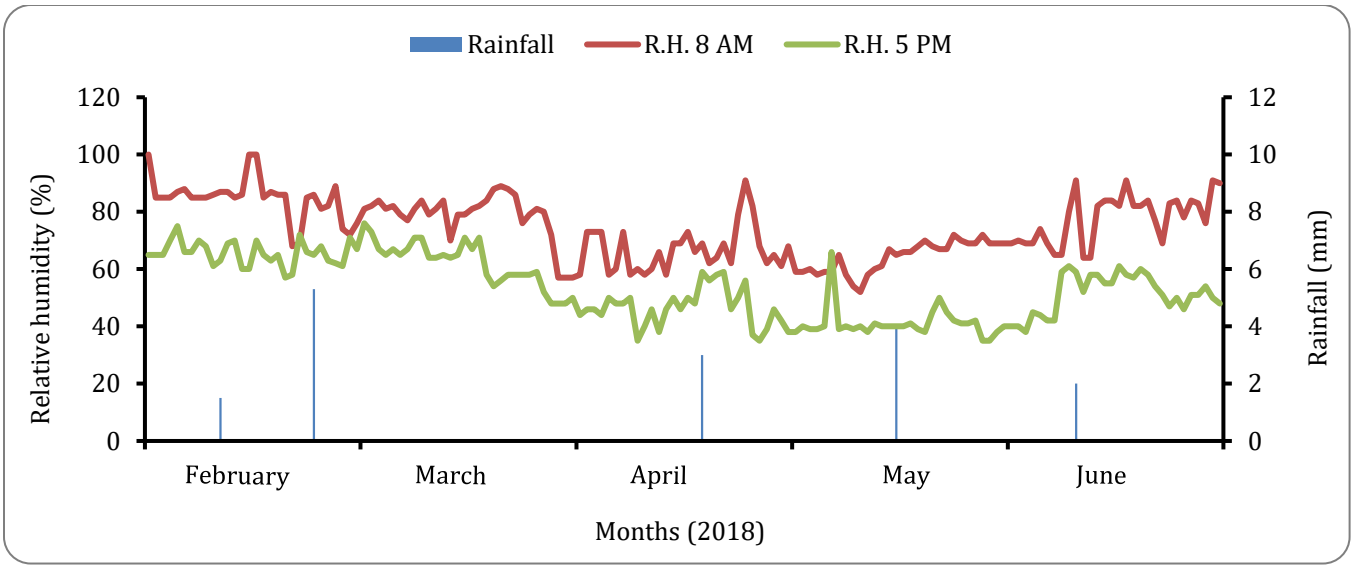

Figure 2. Meteorological data of relative humidity and rainfall recorded,

RESULTS

Application of moringa leaf extracts foliar spray and irrigation levels significantly affect the growth, yield and quality attributes of sunflower (table 2). The interaction of moringa leaf extract with irrigation levels also significantly affected on plant height, head diameter, stem diameter, number of achenes per head, 100-achenes weight, oil yield, protein yield, achenes yield, straw yield and biological yield. The interaction of foliar application of moringa leaf extract an irrigation regime was also significant on quality parameters like oil contents and protein contents on crop growth rate (CGR) and leaf area duration (LAD).

Foliar application of moringa leaf extract (MLE) at F3 (K+H+S) stage with I_0 (normal irrigation significantly ($p \leq 0.05$) increased the plant height (257.33), head diameter (22.17cm), stem diameter (4.43cm), number of aches per head (1030.30) and 1000-achenes weight (49.7g) as compared to F_0 (no spray) with I_3 where minimum plant height (225.33), head diameter (19.57cm), stem diameter (4.03cm), number of aches per head (1001.7) and 1000-achenes weight (47.8g) was observed.

Yield attributes were ranged from Oil yield (1449.9-1322.7kg/ha), achenes yield (3474-3306.7kg/ha), straw yield (7505-7171kg/ha) and biological yield (10979-10478.6kg/ha) at F_3 with I_0 and F_0 with I_3 respectively (table 2).

Like growth and yield parameters, quality and physiological parameters were also affected by foliar application of moringa leaf extract (MLE) and irrigation regimes. Maximum oil contents (41.7%), protein contents (20.8%), harvest index (31.64), leaf area duration (171.5) and crop growth rate (15.89) was observed at F_3 (K+H+S) stage and I_0 treatment. While, minimum oil contents (40%), protein contents (19.47%), leaf area duration (134.2) and crop growth rate (12.54) was observed in F_0 (no sprat) and minimum harvest index (31.26) was observed at F_0 (K+H) and I_3treatment.

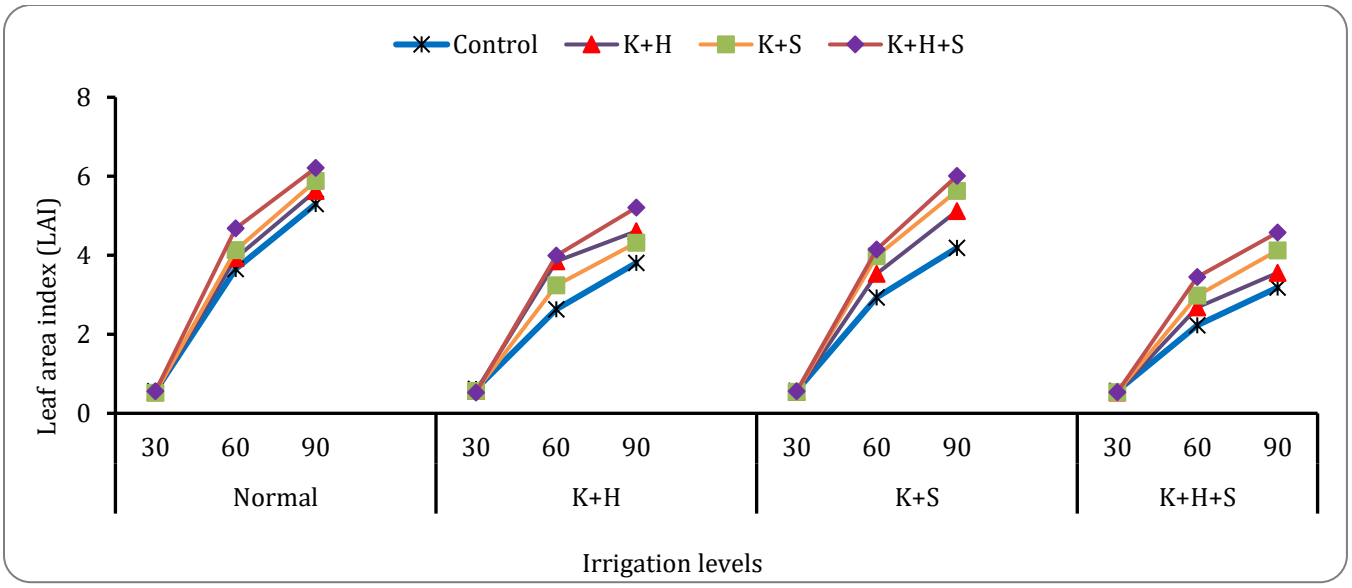

Figure 3. Effect of foliar application of moringa leaf extract (MLE) on Leaf area index of sunflower under different irrigation regimes.

K + H = knee height + heading; K + S= knee height + seed development; K + H +S = knee height + heading + seed development

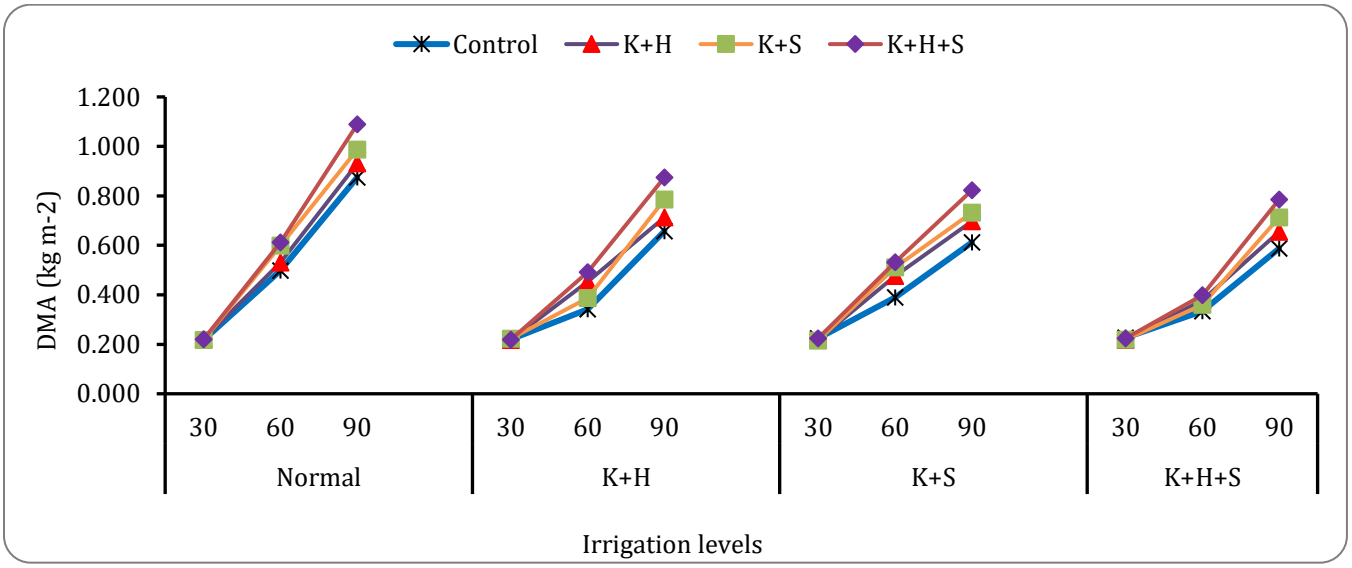

Figure 4. Total dry matter of sunflower as the affected by foliar applied moringa leaf extract under various irrigation regimes

K + H = knee height + heading; K + S= knee height + seed development; K + H +S = knee height + heading + seed development

DISCUSSION

In the world, the sunflower is an important oilseed crop and ranked as 4th after soybean, rapeseed and peanut . In Pakistan, it is also cultivated during spring and autumn season as an oilseed crop. Sunflower achenes contain 40-45 % oil of high quality . In crop production, water stress is a great environmental limitation and it depends on some factors like rainfall distribution and occurrences, soil water storing capacity and evaporative demand . Sunflower is sensitive to heat and water stress at flowering and achenes filling stage due to inadequacy in regulating the transpiration rate and leaf expression

under deficient availability of soil moisture . In minimum rainfall, region yield is eventually decreasing significantly due to a reduction in soil moisture which causes the leaf wilting . reported that oil quality and achenes yield of sunflower is reducing greatly due to moisture stress. Plant height reduces in case of drought stress at vegetative stage . reported that in sunflower severe drought stress reduces net assimilation rate (NAR) which results in stunted growth. In sunflower drought stress is responsible for the reduction in chlorophyll synthesis which results in less production of photoassimilates (. , and reported that when drought stress is subjected at the vegetative stage in sunflower, it effects on carbon capturing and physiological parameters. Leaf area duration (LAD) and crop growth rate (CGR) is reduced when drought stress is subjected at critical stages of sunflower-like knee height, seed development and at heading stage.

Plant growth can be enhanced by exogenous application of plant growth regulators (PGR) like auxins, zeatin, cytokinin and vitamin-c. But the artificial application of these compounds is very costly. The scientist start work on exploring natural plant extract that contains these plant growth regulators which can improve the crop yield. A substantial amount of zeatin is available in extract of moringa leaves . Moringa leaf extract (MLE) not only improve plant growth and development but it also improves crop yield . Culver reported that foliar application of moringa leaf extract (MLE) improves plant height. concluded that maximum height in sunflower is the result of zeatin present in the moringa leaf extract. Presence of cytokinins, acrobats, phenol and minerals like calcium and potassium in the extract of moringa leaf is responsible for the enhancement of crop growth rate in sunflower. Increase in leaf area index under drought or normal condition might be responsible due to presence of growth enhancer in moringa leaf extract .

CONCLUSION

It was concluded that drought stress in sunflower at knee height+ heading+ seed development is most detrimental for growth and development. Also, three foliar application of moringa leaf extract (MLE) at height+ heading+ seed development under normal irrigation boost up the crop yield and high quality of seed can be obtained. Furthermore, more research is recommended on the exogenous application of moringa leaf extract (MLE) to confirm the results.

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Table 2. Response of foliar application of moringa leaf extract (MLE) to growth, yield, quality and physiological parameters of sunflower under different irrigation regimes.

Parameters	F_0				F_1				F_2				F_3			
	I_0	I_1	I_2	I_3	I_0	I_1	I_2	I_3	I_0	I_1	I_2	I_3	I_0	I_1	I_2	I_3
Plant height (cm)	225.33c	163.67gh	178.0e	136.67i	252.33a	178.33e	187.33d	157.0h	234.67b	170.0ef	193.33d	159.0h	257.33a	191.67d	165.67fg	174.33ef
Stem diameter (cm)	4.03c	3.26gh	3.66e	2.6k	4.23b	3.16hi	3.8d	2.8j	4.33ab	3.33g	3.9d	3.1i	4.43a	3.46f	4.1c	3.23gh
Head diameter (cm)	19.57de	17.23h	16.94hi	15.57j	19.90cd	18.53fg	18.70fg	16.77hi	21.0b	18.10g	19.07ef	16.57i	22.17a	20.47bc	19.90cd	19.0ef
Number of achenes per head	1001.7a	803.3d	741.0fg	681.7h	1014.7a	919.3b	803.0de	709.0gh	1018.7a	862.3c	760.0ef	699.0gh	1030.3a	932.7b	833.3cd	821.0cd
1000 achene weight (g)	47.80cd	44.0ij	43.50jk	42.0l	48.4bc	46.2fg	45.1h	42.4l	48.7b	46.5ef	45.9g	43.2k	49.7a	47.1de	46.9ef	44.5hi
Oil yield Kg/ha	1322.7c	1031.9g	1036.3g	920.8i	1366.2b	1135.5f	1181.8e	966.0h	1385.5b	1142.2f	1196.9e	980.0h	1450.0a	1264.1d	1251.2d	1031.7g
Achene yield Kg/ha	3306.7b	2786.3f	2803.3f	2536.3h	3357b	2986de	3048.7d	2599h	3365.7b	2964.3e	3055.7d	2608.7gh	3474a	3194.3c	3148.7c	26.77.3g
Straw yield Kg/ha	7172	6179	6093.7	5653	7383	6366.7	6276	5855	7356	6536.7	6282	5771	7505	6518.3	6413.7	5927
Biological yield Kg/ha	10478.6c	8965.3g	8897g	8209.3j	10740b	9352.7f	9324.7f	8454i	10721.6b	9501e	9338f	8379.7i	10979a	9712.7d	9562.3e	8604.3h
Harvest index	31.56bc	31.23bc	31.51bc	30.84bc	31.26bc	32.07b	32.7a	30.74c	31.39bc	31.2bc	32.72a	31.13bc	31.64b	32.8a	32.93a	31.0bc
Oil contents (%)	40c	37.0j	36.9j	36.3k	40.7b	38.0gh	38.7ef	37.2ij	41.2b	38.5f	39.2de	37.6hi	41.7a	39.6cd	39.7c	38.5fg
Protein contents (%)	19.47de	16.67jk	16.8jk	16.27k	20.07bc	18.2hi	18.67gh	16.77jk	20.14b	19fg	19.1ef	16.94j	20.8a	19.57cd	19.9bc	17.34i
CGR	12.54c	10.50de	7.40gh	8.47fg	13.32bc	8.66fg	7.35h	9.20ef	14.03b	13.27bc	7.36h	11.76cd	15.89a	12.77bc	9.68ef	12.93bc
LAD	134.2d	96.6i	106.8h	81.1j	143.4c	126.9e	137.8d	93.6i	150.2b	113.40g	128.1e	106.6h	171.5a	138.0d	152.4b	120.4f